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Differential Stimulus Control of Drug-seeking: Multimodal Reinstatement

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Abstract

In animal models of substance-use disorder, individuals that repeatedly self-administer drugs of abuse have long lasting neuronal adaptations that do not occur ostensibly in control animals only exposed to natural reinforcers (e.g. food). Because any treatment for substance-use disorder will be given to individuals with drug-taking histories, adequate dissociation of the specific neurobehavioral mechanisms underlying drug reinforcement, natural reinforcement, and their associated cue effects, requires an experimental model that exposes individuals to both reinforcer conditions, along with their associated stimuli. Furthermore, contingent stimuli that reinforce drug seeking through second-order relationships may produce reinstatement of drug seeking through different neurobehavioral means than non-contingent exposure to stimuli that signal the availability of a drug reinforcer, effectively producing different modes of stimulus-induced reinstatement. Toward experimental isolation of the above relationships, herein we used a within-session multiple schedule of reinforcement containing both discriminative (S^D) and conditioned (CS) stimuli to study stimulus control of drug- and food-taking behavior, along with how these functionally-distinct cues may differentially reinstate drug- and food-seeking behavior within a single animal. We demonstrate specific stimulus control over drug and food taking; furthermore, we demonstrate that the same stimulus (i.e. cue light) operating as a S^D or CS produced differential reinstatement of drug- and food-taking behavior. The results suggest that contingent CSs and non-contingent S^D s produce reinstatement through different neurobehavioral processes, and within-session multiple schedules can be used to study different modes of specific stimulus control over drug and food seeking in a single animal with both a drug- and food-taking history.

Keywords

Cocaine; Conditioned Stimulus; Discriminative Stimulus; Methamphetamine; Multiple Schedule; Reinstatement

INTRODUCTION

In preclinical research, a neutral stimulus can function as a conditioned stimulus (CS) after being paired consistently and contiguously with a drug infusion (most frequently after an operant response), whereas a neutral stimulus can also come to function as a discriminative

stimulus (S^D) if it specifically signals drug availability (e.g. a light above a lever ‘signals’ that pressing the lever will result in drug delivery). Thus, the function of drug-associated stimuli is dependent upon its relationship with the drug reinforcer. There is evidence that these functionally distinct stimuli can both come to control drug-taking (See et al. 1999; Schenk and Partridge 2001; Weiss 2003) and drug-seeking behavior (Weiss et al. 2001; Bossert et al. 2013), but they appear to do so through different means. For example, evidence suggests that a drug infusion-paired CS does not promote reinstatement if it presented non-contingently; however, the same stimulus does promote reinstatement if it is presented contingent upon lever pressing (Grimm et al., 2000). The necessity of contingent presentation of the infusion-paired CS on reinstatement of drug seeking suggests that the CS maintains behavior by functioning as a conditioned reinforcer. Additionally, contingent presentation of an infusion-paired CS maintains behavior far more effectively than contingent presentation of a drug-predictive S^D , while the opposite is true for drug-predictive S^D s (Grimm et al. 2000; Di Ciano and Everitt, 2003), suggesting the S^D modulates operant drug-seeking behavior by informing the animal as to when the drug-seeking response is likely to be reinforced by the drug reinforcer. Finally, there are individual differences in the efficacy of a CS or S^D to reinstate drug-seeking behavior. Data suggests that animals that sign-track show increased reinstatement to a contingent infusion-paired CS compared to animals that goal-track (Saunders and Robinson, 2010). Conversely, a non-contingent context S^D reinstates cocaine-seeking behavior to a greater extent in animals that goal-track compared to those that sign-track (Saunders et al., 2012). Thus, infusion-paired CSs and drug-predictive S^D s represent two distinct modes of stimulus control over drug-taking and -seeking behavior that are mediated by different neurobehavioral mechanisms.

In addition to differences in stimulus function, to date, most preclinical studies attempt to study drug-specific neural changes in animals that only self-administer drug, while controls only self-administer food, water, or receive yoked saline (Cunningham et al., 2015; Huff & LaLumiere, 2015; Saddoris et al., 2016). However, controls likely do not have the same neuronal adaptations as rats with a history of drug taking, limiting the ability to study drug-specific neural adaptations on which to develop pharmacotherapies that specifically decrease drug-taking behavior. This is particularly important when considering that no persons with substance-use disorder are drug naïve, and they exhibit a wide array of behavior beyond drug taking that is maintained by non-drug reinforcement. Thus, in order to adequately study drug-taking and drug-seeking behavior, including drug-specific neural adaptations, a design must be used that exposes the same individual to drugs, natural rewards, and their associated cues.

Multiple schedules of reinforcement allow for the study of drug and food reinforcement, as well as their associated stimulus effects within a single individual (Weissenborn et al., 1995; Weissenborn et al., 1996; Carelli et al., 2000; Stairs et al., 2010). Previous studies have used multiple schedules in order to study primary reinforcers as well as the effects of associated CSs and S^D s (Weiss et al., 2000; Weiss et al., 2001; Weiss et al., 2003; Kearns & Weiss, 2005; Kearns & Weiss, 2007; Weiss et al., 2007). However, to our knowledge, a systematic investigation into how CSs vs. S^D s differentially control drug- and food-taking behavior and reinstate drug- and food-seeking behavior has yet to be done. Herein, we use a within-

session multiple schedule procedure in order to examine drug (methamphetamine and cocaine) vs. food reinforcement, how their associated stimuli (CS and S^D) may differentially function to specifically control drug- and food-taking, and we specifically reinstate drug- or food-seeking behavior using both S^Ds (non-contingent presentation) and CSs (contingent presentation).

MATERIALS AND METHODS

Animals

Twenty-eight adult male Sprague-Dawley rats (Harlan, Inc.; Indianapolis, IN, USA) weighing approximately 250–300 g were used for experimentation. Rats were individually housed in a temperature-controlled environment on a 12:12 hour light:dark cycle with lights on at 0600 hours. All rats were acclimated to the colony room and handled one week before any experimentation began. All rats had *ad libitum* access to water and food during the experiment proper. The Institutional Animal Care and Use Committee at the University of Kentucky approved all experimental protocols.

Drugs

(+)-Methamphetamine hydrochloride (METH; Sigma Aldrich, St. Louis, MO) and cocaine hydrochloride (COC; NIDA, Rockville, MD) were prepared in sterile 0.9% saline for self-administration. METH was self-administered at 0.05 mg/kg/infusion and COC was self-administered at 0.3 mg/kg/infusion (Green et al., 2010; Beckmann et al., 2012) based on weight.

Apparatus

Experiments were conducted in an operant conditioning chamber (ENV-008, Med Associates) housed within a sound-attenuating compartment (ENV-018M, Med Associates). Each chamber was connected to a computer (SG-502, Med Associates) and ran using MED-PC. Each operant chamber contained a 5.1 cm × 5.1 cm recessed food receptacle (ENV-200R2MA) on the front response panel with two retractable levers on either side (ENV-122CM; 6 cm above metal rod floor). Above each lever was one white cue light (ENV-221M; mounted 4.1 cm above each lever). Located above the top left cue light was a Sonalert tone (ENV-223 AM) and located above the top right cue light was another Sonalert tone (ENV-223 HAM). A house light (ENV-227M) was placed 17 cm above the metal floor in the middle of the back wall. Food pellets (45 mg, Dustless Precision Pellets; Bio Serv) were delivered via a dispenser (ENV-203M-45) placed behind the food receptacle. All drugs were self-administered via a syringe pump (PHM-100) located outside of the sound-attenuating chamber. The drugs were pumped through a watertight swivel attached via tygon tubing that was attached to a head-mounted cannula.

Initial Training and Surgery

All animals were trained to respond on a lever for food, counter balanced for side, according to a fixed-ratio 1 (FR1) for two days with no stimuli. Rats were then surgically implanted with a chronic indwelling jugular catheter and allowed to recover for 7 days. Upon recovery, rats were trained on an FR5 for drug in the presence of the drug S^D. Specifically, rats were

split into five groups with two groups allowed to self-administer METH and three groups allowed to self-administer COC. Within each drug group there were two different stimulus conditions. One group of rats self-administered METH and another group of rats self-administered COC with the cue light above the lever serving as the drug S^D and a compound stimulus of a solid tone and house light serving as the infusion CS where the compound CS signaled the drug infusion period (5.9 s). From this point forward we will refer to these two groups of rats as the METH S^D Light/CS House group (Fig. 1A) and the COC S^D Light/CS House group (Fig. 1A), respectively. Another group of rats self-administered METH and another group of rats self-administered COC with the compound stimulus of a solid tone and house light serving as the drug S^D and the cue light above the drug lever serving as the infusion-associated CS. From this point forward we will refer to these two groups as the METH S^D House\CS Light group (Fig. 1B) and the COC S^D House\CS Light group (Fig. 1B), respectively (note that between the two environmental conditions the CS and S^D were counter balanced; the active and inactive levers were also counterbalanced across all groups). The fifth group of rats was similar to the COC S^D Light/CS House group however they were sated on food pellets before reinstatement; we refer to this group as the COC S^D Light/CS House Prefeed group (Fig 1A). Once animals' drug-taking behavior was stable they were then trained on the multiple schedule.

Multiple Schedule Training

All rats were trained on a multiple schedule procedure for at least 14 days. Stability was defined as at least 10 reinforcers earned in each component, an active-inactive lever ratio of 2:1, and less than 20% variability in each measure across 3 consecutive days after at least 14 days of training (this is referred to as baseline responding henceforth). The procedure consisted of 12 alternating 10-minute components per session (with the first component being randomly selected) of either drug (6 components) or food (6 components) availability separated by 2-minute inter-component intervals (ICI), a period between components where all stimuli and manipulandum were off and no reinforcers could be earned. The S^D and CS stimulus conditions for the drug components were as described above. The S^D signaling food components for the METH S^D Light/CS House group (Fig. 1A), the COC S^D Light/CS House group (Fig. 1A), and the COC S^D Light/CS House Prefeed group (Fig. 1A) was the cue light above the food lever, and the CS (5.9 s) signaling food pellet delivery was as a blinking house light. For the METH S^D House\CS Light group (Fig. 1B) and COC S^D House\CS Light group (Fig. 1B), the food S^D was a blinking house light and the pellet-associated CS was the cue light above the food lever (5.9 s). Note that the food lever and associated stimuli were opposite of the drug lever. Again, there were five groups in total (see Table 1).

Extinction

After stable responding (see above) in both the drug and food components of the multiple schedule animals were put through a 14-day extinction period. Specifically, the METH S^D Light/CS House group (Fig. 1A), COC S^D Light/CS House group (Fig. 1A), and COC S^D Light/CS House Prefeed group (Fig. 1A) underwent extinction in the absence of any S^D or CS (for non-contingent S^D reinstatement, see below; Table 1). The METH S^D House\CS Light group (Fig. 1B) and COC S^D House\CS Light group (Fig. 1B) underwent extinction in

the presence of the S^D but the absence of the CS (for contingent CS reinstatement, see below; Table 1).

Discriminative Stimulus and Conditioned Stimulus Reinstatement

The METH S^D Light/CS House group (Fig. 1A), COC S^D Light/CS House group (Fig. 1A), and COC S^D Light/CS House Prefeed group (Fig. 1A) were exposed non-contingently to both the drug and food S^D s in the same component structure as in baseline training but without reinforcement (non-contingent S^D reinstatement; Table 1). Note that the COC S^D Light/CS House Prefeed group (Fig. 1A) were sated on food pellets one hour prior to reinstatement in an attempt to decrease food reinstatement to further show that we had stimulus control in our multiple schedule procedure and that we could manipulate behavior in a reinforcer specific fashion. None of the animals in these experiments were food restricted; the prefeed group was simply given 1-hour unlimited access to the food pellets used for food reinforcement before the reinstatement session. The METH S^D House\CS Light group (Fig. 1B) and COC S^D House\CS Light group (Fig. 1B) were exposed to each S^D (drug and food) non-contingently in the same component structure as in the multiple schedule, however responses on the active lever resulted in presentation of the drug or food-paired CS (contingent CS reinstatement; Table 1). Note that the S^D was present during extinction so that we could isolate the effects of the CS during reinstatement.

Data Analysis

All data were analyzed using JMP Pro 12.1.0 statistical software and all graphs were made using Prism GraphPad (version 7.0b). Extinction data was analyzed using linear mixed-effects modeling with subject as a random factor and lever as a fixed, within-subjects factor. Baseline and reinstatement data were analyzed using linear mixed-effects modeling with subject as a random factor and lever and stimulus as fixed, within-subjects factors. All interactions were probed using the Bonferroni correction.

RESULTS

Methamphetamine Baseline Behavior, Extinction, and S^D Reinstatement

Figure 2A shows baseline responding as a function of reinforcer lever in the presence of the S^D for either METH or food for the METH S^D Light\CS House group that were in the non-contingent S^D condition (Table 1). There was a significant lever $\times$ stimulus interaction [$F(1, 5) = 30.02$, $p = 0.003$], with more lever responses seen on the food lever than the METH lever during the presentation of the food S^D , more responses observed on the food lever in the presence of the food S^D compared to the food lever during the presentation of the METH S^D , more responses on the METH lever than the food lever during the METH S^D , and more responses on the METH lever during the METH S^D than during the food S^D .

Figure 2B shows the extinction curves for both METH and food for the METH S^D Light\CS House group that were in the non-contingent S^D condition. Note again that for this group of rats no stimuli were present during extinction. There was a main effect of time such that responses decreased as a function of session [$F(1, 5) = 32.97$, $p = 0.002$].

Figure 2C shows the number of reinstated responses for the METH S^D Light\CS House rats with non-contingent presentation of both the METH and food S^D, relative to the last day of extinction. There was a significant main effect of stimulus [$F(1, 5) = 12.73, p = 0.02$], with more responses seen during the presentation of the METH and food S^D compared to the extinction baseline. Finally, there were no significant increases in responding on the food lever during the METH S^D (9.12 ± 2.34) or responding on the METH lever during the food S^D (4.32 ± 2.2), relative to extinction baseline.

Methamphetamine Baseline Behavior, Extinction, and CS Reinstatement

Figure 2D shows baseline responding as a function of reinforcer lever in the presence of the S^D for either METH or food during the METH S^D House\CS Light group for the contingent CS condition (Table 1). There was a significant lever $\times$ stimulus interaction [$F(1, 4) = 25.01, p = 0.008$], with more lever responses seen on the food lever than the METH lever during the presentation of the food S^D, more responses observed on the food lever in the presence of the food S^D compared to the food lever during the presentation of the METH S^D, more responses on the METH lever than the food lever during the METH S^D, and more responses on the METH lever during the METH S^D than during the food S^D.

Figure 2E shows the extinction curves for both METH and food for the METH S^D House\CS Light group that were in the contingent CS condition. Note again that for this group of rats the S^D was present during extinction in order to isolate the effect of the CS during reinstatement. There was a main effect of time such that responses decreased as a function of session [$F(1, 4) = 16.88, p = 0.02$].

Figure 2F shows the number of reinstated responses for the METH S^D House\CS Light rats with contingent presentation of the METH or food CS compared to the last day of extinction. There was a main effect of stimulus [$F(1, 4) = 9.42, p = 0.04$], with more responses observed in the presence of the METH and food CS compared to extinction baseline. Finally, there were no significant increases in responding on the food lever during the METH S^D (5.7 ± 2.94) or responding on the METH lever during the food S^D (7.22 ± 1.96), relative to extinction baseline.

Cocaine Baseline Behavior, Extinction, and S^D Reinstatement

Figure 3A shows baseline responding as a function of reinforcer lever in the presence of the S^D for either COC or food during the COC S^D Light\CS House group for the non-contingent S^D condition (Table 1). There was a significant lever $\times$ stimulus interaction [$F(1, 4) = 344.77, p < 0.0001$], with more lever responses seen on the COC lever than the food lever during the COC S^D, more responses seen on the COC lever in the presence of the COC S^D compared to responses on the COC lever in the presence of the food S^D, more responses on the food lever in the presences of the food S^D compared to responses on the food lever in the presences of the COC S^D, and more responses on the food lever in the presences of the food S^D compared to responses on the COC lever in the presence of the food S^D.

Figure 3B shows the extinction curves for both COC and food for the COC S^D Light\CS House group that were in the non-contingent S^D condition. Note again that for this group of rats no stimuli were present during extinction. There was a main effect of time such that

responses decreased as a function of session [$F(1, 4) = 482.9$, $p < 0.0001$]. There was also a lever $\times$ extinction day interaction such that baseline responding for food was higher than that for COC [$F(1, 4) = 7.81$, $p = 0.049$].

Figure 3C shows the number of reinstated responses for the COC S^D Light\CS House rats during non-contingent presentation of the COC and food S^D , relative to the last day of extinction. There was a main effect of lever [$F(1, 4) = 13.06$, $p = 0.02$], with more responses observed on the food lever compared to the COC lever. There was also a main effect of stimulus [$F(1, 4) = 85.87$, $p = 0.0008$], with more responses seen in the presence of the COC and food S^D , in relation to the extinction baseline. Finally, there were no significant increases in responding on the food lever during the COC S^D (6.50 ± 3.7) or responding on the COC lever during the food S^D (5.43 ± 2.11), relative to extinction baseline.

Cocaine Baseline Behavior, Extinction, and CS Reinstatement

Figure 3D shows baseline responding as a function of reinforcer lever in the presence of the S^D for either the COC or food lever during the COC S^D House\CS Light group for the contingent CS condition (Table 1). There was a significant lever $\times$ stimulus interaction [$F(1, 5) = 146.69$, $p < 0.0001$], with more lever presses seen on the COC lever than the food lever during the presentation of the COC S^D , more lever presses seen on the COC lever during the COC S^D compared to COC lever presses during the food S^D , more lever presses seen on the food lever than the COC lever in the presence of the food S^D , and more lever presses seen on the food lever during the presentation of the food S^D compared to food lever presses during the presentation of COC S^D .

Figure 3E shows the extinction curves for both COC and food for the COC S^D House\CS Light group that were in the contingent CS condition. Note again that for this group of rats the S^D was present during extinction in order to isolate the effect of the CS during reinstatement. There was a main effect of time such that responses decreased as a function of session [$F(1, 4) = 418.98$, $p = 0.0001$].

Figure 3F shows the number of reinstated responses for that COC S^D House\CS Light rats with contingent exposure to either the COC or food CS, relative to the last day of extinction. There was a significant lever $\times$ stimulus interaction [$F(1, 5) = 7.19$, $p = 0.04$], with more responses seen on the COC lever during contingent presentation of the COC CS compared to the last day of COC extinction and more responses seen on the COC lever during the contingent presentation of the COC CS compared to the food extinction baseline. Finally, there were no significant increases in responding on the food lever during the COC S^D (7.31 ± 2.77) or responding on the COC lever during the food S^D (8.34 ± 2.5), relative to extinction baseline.

Cocaine Baseline Behavior, Extinction, and S^D Reinstatement: Prefeed Condition

Figure 3G shows baseline responding as a function of reinforcer lever in the presence of the S^D for either COC or food during the COC S^D Light\CS House Prefeed group for the non-contingent S^D condition (Table 1). There was a significant lever $\times$ stimulus interaction [$F(1, 5) = 58.77$, $p = 0.0006$], with more lever responses seen on the COC lever than the food lever during the COC S^D , more responses seen on the COC lever in the presence of the COC

S^D compared to responses on the COC lever in the presence of the food S^D , more responses on the food lever in the presence of the food S^D compared to responses on the food lever in the presences of the COC S^D , and with more responses on the food lever in the presence of the food S^D compared to responses on the COC lever in the presence of the food S^D .

Figure 3H shows the extinction curves for both COC and food for the COC S^D Light\CS House Prefeed group that were in the non-contingent S^D condition. Note again that for this group of rats no stimuli were present during extinction. There was a main effect of time such that responses decreased as a function of session [$F(1, 5) = 91.91, p = 0.0002$].

Figure 3I shows the number of reinstated responses for the COC S^D Light\CS House Prefeed rats with non-contingent presentation of the COC and food S^D , after being sated on food pellets, relative to the last day of extinction. There was a significant lever $\times$ stimulus interaction [$F(1, 5) = 50.20, p = 0.0009$], with more responses seen on the COC lever in the presence of the COC S^D compared to responses on the food lever during the presentation of the food S^D , more responses on the COC lever during the presentation of the COC S^D compare to the COC extinction baseline, and more responses on the COC lever in the presence of the COC S^D compared to the food extinction baseline. Finally, there were no significant increases in responding on the food lever during the COC S^D (5.23 ± 1.9) or responding on the COC lever during the food S^D (6.87 ± 2.78), relative to extinction baseline.

In summary, our data show that we obtained control of all baseline behavior and achieved reinstatement of all drug and food behavior with the exception of food reinstatement for the COC S^D House\CS Light group in the contingent CS reinstatement condition (Figure 3F). Further, we showed that we could decrease the responses for food during non-contingent S^D reinstatement in the prefeed group, while not affecting COC reinstatement, by allowing rats to access to the operant chamber food pellets in their home cage 1 hour prior to reinstatement (Figure 3I).

DISCUSSION

While others have used multiple schedules to gain stimulus control over drug- and food-taking behavior (or drug/food-taking and extinction) by using S^D s (Weiss et al., 2000; Weiss et al., 2001; Weiss et al., 2003; Kearns & Weiss, 2005; Weiss et al., 2007) or CSs (Weissenborn et al., 1995; Weissenborn et al., 1996; Carelli et al., 2000), the present study adds to this previous research by demonstrating that a drug/food multiple schedule using both S^D s and CSs can be used to not only gain stimulus control of drug- and food-taking behavior (as in the baseline responding reported here), but also to reinstate previously extinguished behavior within a single animal using both a non-contingent S^D s and a contingent CSs (i.e., multimodal reinstatement). The present results also demonstrate reinforcer-specific stimulus control over reinstatement, as shown by decreased responding for food during food reinstatement in the COC S^D Light/CS House Prefeed group that were sated on food pellets prior to reinstatement (Figure 3I). Of special interest is the fact that the same stimulus (i.e. cue light above the lever) was used to function as a CS or a S^D , allowing for the isolation of stimulus function, which was replicated multiple times across two

different stimulant drugs. Thus, the results presented here illustrate differential modes of stimulus control of both taking and seeking behavior for drug and non-drug reinforcers within a single animal, specifically isolating the functional role of these stimuli in drug-associated behavior.

The within session multiple schedule procedure presented here is also amenable to isolating drug-specific neural adaptations that occur during drug-taking and drug-seeking behavior. For example, if a researcher is interested in isolating a particular brain region or neuronal tract that may contribute to drug-taking behavior they could train animals on the present procedure to baseline. The researcher could then use optogenetics or chemogenetics to turn that subset of neurons 'on' or 'off' while animals perform in the multiple schedule. By doing this, the researcher could then determine if the responses for drug and food change during the session in relation to the neuronal manipulation (the ideal data being a decrease in drug responding with no change in food responding, within the same system). In this way, one could then begin to isolate and dissociate certain neural mechanisms that contribute specifically to drug-taking behavior and specific associated stimulus control. Further, following a similar logic, a researcher could use this procedure to also study how drug and food CSs and S^Ds engage specific neural circuits and work to specifically promote and maintain drug- or food- seeking behavior. Thus, by using this procedure one can gain a deeper understanding of the aforementioned neural processes, which may lead to the development of better pharmacotherapies that specifically decrease drug-taking and seeking behavior while not affecting other reinforced behavior.

It is worth noting that significant food reinstatement for the COC S^D House\CS Light group in the contingent CS condition was not observed. Although contingent CS-induced reinstatement of food seeking was observed within the METH S^D House\CS Light group, it is possible that we did not obtain cue-induced reinstatement of food in the COC contingent reinstatement group due to the fact that we used the cue light above the lever as the CS. When a food pellet is delivered, animals have to move away from the CS to approach the food receptacle in order to retrieve their pellet; thus, it is possible that a strong CS-food association was not acquired. Conversely, considering rats receive drug via an indwelling catheter and do not have to exhibit any approach behavior to consume the drug reinforcer, it is possible that the drug cue light CS promotes a stronger association. Thus, if a more disperse CS had been used (e.g., tone, house light), or if the CS were located proximal to the location of food consumption (e.g., a magazine light located in the food receptacle), we may have produced a stronger CS-food relationship and associated conditioned reinforcement, making contingent CS-induced reinstatement of food seeking more consistent. Future studies should attempt to better equate the CS-US associative strength for drug and food CSs when attempting to reinstate drug- and food-seeking behavior via contingent access to each CS.

A widely used procedure used for studying S^Ds in preclinical research on stimulus control of drug seeking is context-induced reinstatement. The most common procedure trains animals to self-administer drug in one context (A), then animals are placed in another context where an operant no longer produces drug and responding is extinguished (B). After extinction, animals are then placed back into context A where the context alone increases responding,

even though there are no programmed consequences (Crombag & Shaham, 2002; Lasseter et al., 2011; Lasseter et al., 2014). In this example, animals learn that cocaine is available upon a specified operant in context A but not in context B, and when re-exposed to context A, where no extinction had been trained in context A prior to re-exposure, animals resume responding for cocaine; thus, context A serves functionally as a S^D for cocaine reinforcement. Unfortunately, the use of context to study the neurobehavioral processes that underlie S^D -induced reinstatement makes it difficult to dissociate the effects of using context as a stimulus from its functional properties of serving as a S^D . Because context is typically omnipresent, it is procedurally difficult to pair context with the infusion of drug alone (as is done with a punctate light stimulus, for example), making it difficult to train context as a contingent infusion-associated CS. Thus, it is difficult to determine the neurobehavioral differences between context functioning as a CS vs. context functioning as an S^D in the stimulus control over drug-seeking behavior. For example, the dorsal hippocampus and the basolateral amygdala are important in non-contingent context-induced reinstatement of drug seeking, whereas the basolateral amygdala and medial prefrontal cortex are important for non-contingent S^D -induced reinstatement via a punctate stimulus (Ciccocioppo et al., 2001; Fuchs et al., 2005). Further, there is evidence that inactivation of the nucleus accumbens core may prevent contingent CS-induced reinstatement, whereas inhibition of the nucleus accumbens core or shell prevents non-contingent contextual reinstatement (Fuchs et al., 2004; Fuchs et al., 2008). Thus, considering the above it is impossible to know if the observed neural differences between a punctate and contextual stimulus are due to the functional differences between these stimuli or are due to the difference in how punctate vs. contextual stimuli affect different neuronal populations. The procedure used here has the capacity to isolate these different neurobehavioral mechanisms because it allows the same punctate stimulus to be used as either a CS or S^D . Thus, any stimulus differences observed using the multiple schedule procedure can be attributed to their functional differences, rather than physical differences between the stimuli.

Generally speaking, the preclinical literature on stimulus control of drug seeking is dominated by the cue-induced reinstatement model that is reliant upon contingent presentation of an infusion-paired CS, whereas human clinical research is dominated primarily by non-contingent stimulus presentation, such as in cue-induced craving and attentional bias studies (Sinha & Li, 2007; Field & Cox, 2008; Bossert et al., 2013; Lasseter et al., 2014). Given the lack of reinstatement associated with non-contingent presentation of infusion-paired CSs (Grimm et al. 2000) and the lack of drug seeking associated with contingent presentation of S^D s, the translational generality of the conditioned reinforcement model of reinstatement is questionable. Clearly, non-contingent presentation of drug-associated cues is effective in clinical research; thus, to foster greater translational efficacy, it will be important that future work in preclinical models focus on stimulus control of drug seeking via non-contingent stimuli, like drug-predictive S^D s. Additionally, in order to better understand how drug-associated stimuli might be functioning as conditioned reinforcers, a focus on contingent access to drug-associated stimuli in clinical studies would help corroborate the existing preclinical research. Collectively, this cross-translational approach may help better identify effective anti-relapse therapies.

In conclusion, the present results suggest that a drug/food multiple schedule can be used not only to pinpoint specific modes of stimulus control, but can also be used to further understand how these functionally distinct drug and food cues work to specifically modulate drug- and food-seeking behavior. Thus, utilizing drug/food multiple schedules akin to that proposed herein will aid in the isolation of the neurobehavioral processes that specifically govern drug-taking and drug-seeking behavior, and isolating these specific processes will aid in the future development of both behavioral and pharmacological treatments for substance-use disorder.

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Figure 1. Stimulus Conditions in the Multiple Schedule Procedure for Each Group of Rats

All four sections of the two figures (A-B) represent the intelligence panel of a standard operant chamber. The top two panels represent the S^D for drug and food, respectively. The bottom two panels represent the CS for drug and food, respectively. The active and inactive levers are identified. The different groups of rats are indicated under the figures. (A)

Multiple Schedule for METH S^D Light/CS House, COC S^D Light/CS House, and COC S^D Light/CS House Prefeed Groups. S^D Drug = cue light over respective lever; S^D Food = cue light over respective lever; CS Drug = compound stimulus consisting of solid tone + house light; CS Food = flashing house light. (B) **Multiple Schedule for METH S^D House/CS Light and COC S^D House/CS Light Groups.** S^D Drug = compound stimulus consisting of solid tone + house light; S^D Food = flashing house light; CS Drug = cue light over respective lever; CS Food = cue light over respective lever.

Figure 2.

(A) Baseline Behavior of METH S^D Light\CS House Group for Non-contingent S^D Condition. More food lever presses were seen in the presence of the food S^D compared to the METH S^D; More METH lever presses were seen in the presence of the METH S^D compared to the food S^D ($n = 6$; $*p < 0.05$). **(B) Extinction of METH S^D Light\CS House Group for Non-contingent S^D Condition.** Responses on the METH lever and the food lever decreased as a function of session ($n = 6$; $p < 0.05$). **(C) Reinstatement of METH S^D Light\CS House Group for Non-contingent S^D Exposure.** Both METH and food lever pressing behavior was reinstated by their respective S^D's compared to the extinction baseline

($n = 6$; $*p < 0.05$). **(D) Baseline Behavior of METH S^D House\CS Light Group for Contingent CS Condition.** More METH and food lever presses were seen during the presentation of the METH and food S^Ds, respectively ($n = 5$; $*p < 0.05$). **(E) Extinction of METH S^D House\CS Light Group for Contingent CS Condition.** Responses on the METH and food lever decreased as a function of session ($n = 5$; $p < 0.05$). **(F) Reinstatement of METH S^D House\CS Light Group for Contingent CS Exposure.** METH and food lever pressing behavior was reinstated by the contingent presentation of the METH and food CS, respectively, compared to the extinction baseline ($n = 5$; $*p < 0.05$).

Figure 3.

(A) Baseline Behavior of COC S^D Light\CS House Group for Non-contingent S^D Condition. Statistically more lever presses were seen for COC in the presence of the COC S^D and for food during the presentation of the food S^D ($n = 5$, $*p < 0.05$). **(B) Extinction of COC S^D Light\CS House Group for Non-contingent S^D Condition.** Baseline responding for food was higher than baseline responding for COC. Responses on the COC lever and the food lever decreased as a function of session ($n = 5$; $p < 0.05$). **(C) Reinstatement of COC S^D Light\CS House Group with Non-contingent S^D Exposure.** COC- and food-taking behavior were both reinstated by their respective S^D's compared to extinction baseline;

statistically more food lever presses were observed during the presentation of the food S^D compared to COC lever presses during the COC S^D ($n = 5$, $*p < 0.05$). **(D) Baseline Behavior of COC S^D House\CS Light Group for Contingent CS Condition.** Statistically more lever presses for COC were seen during the presentation of the COC S^D and for food during the presentation of the food S^D ($n = 6$, $*p < 0.05$). **(E) Extinction of COC S^D House\CS Light Group for Contingent CS Condition.** Responses on the COC lever and the food lever decreased as a function of session ($n = 6$; $*p < 0.05$). **(F) Reinstatement of COC S^D House\CS Light Group for Contingent CS Exposure.** COC-taking behavior did reinstate during the presentation of the COC CS; food-taking behavior did not reinstate ($n = 6$, $*p < 0.05$). **(G) Baseline Behavior of COC S^D Light\CS House Prefeed Group for Non-contingent S^D Condition.** Statistically more COC lever presses were observed during the presence of the COC S^D and more lever presses for food were seen during the food S^D ($n = 6$, $*p < 0.05$). **(E) Extinction of COC S^D Light\CS House Prefeed Group for Non-contingent S^D Condition.** Responses on the COC lever and the food lever decreased as a function of session ($n = 6$; $*p < 0.05$). **(I) Reinstatement of COC S^D Light\CS House Prefeed Group for Non-contingent S^D Exposure.** All rats were satiated with food pellets 1 hour before reinstatement. Statistically more COC lever presses were seen in the presence of the COC S^D thus showing reinstatement; food-taking behavior in the presence of the food S^D was decreased during reinstatement compared to COC lever presses in the presence the COC S^D ($n = 6$, $*p < 0.05$).

Table 1

Table 1 shows the number of animals used for each experimental condition as well as the drug used in baseline training, stimuli used in baseline training, type of extinction (S^D present or absent), and type of reinstatement (Non-contingent S^D or contingent CS) used for each experimental group.

Number of Experimental Groups and The Conditions Experienced				
No. of Groups (<i>n</i>)	Drug	Stimulus Condition	Extinction Condition	Reinstatement Condition
1 (6)	METH	S^D Light/CS House	No Stimuli Present	Non-contingent S^D
2 (5)	METH	S^D House/CS Light	S^D Present	Contingent CS
3 (5)	COC	S^D Light/CS House	No Stimuli Present	Non-contingent S^D
4 (6)	COC	S^D House/CS Light	S^D Present	Contingent CS
5 (6)	COC	S^D Light/CS House	No Stimuli Present	^a Non-contingent S^D

^aThis group of rats was sated on food pellets 1 hour prior to reinstatement.